IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Hsing-Pang Hsieh	Art Unit	: 1617
Serial No.	: 10/817,490	Examiner	: Yong Soo Chong
Filed	: April 2, 2004	Conf. No.	: 2330
Title	: Treatment Of Hepatitis C Virus Infection With Sesquiterpene Lactones		

Commissioner for Patents
P.O. Box 1450
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DECLARATION OF SUI-YUAN CHANG UNDER 37 C.F.R. 1.132

I, Sui-Yuan Chang, declare:

1. I hold the positions of Associate Professor at the Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, and Supervisor at the Department of Laboratory Medicine, National Taiwan University Hospital. Prior to these positions, I earned a doctoral degree from the Department of Immunology and Infectious Diseases, Harvard School of Public Health.

2. My current research focuses on genotypic analysis of viruses and therapy of virus infections. Overall, I have studied virus infections for 10 years. .

3. I have reviewed U.S. Serial No. 10/817,490. This application includes claims covering a method of treating hepatitis C virus (HCV) infection with an effective amount of a sesquiterpene lactone compound.

4. I have also reviewed Hwang et al., US Patent 5,905,089 (Hwang) and Baba et al., US Patent 6,123,943 (Baba), both of which are cited in U.S. Serial No. 10/817,490.

Hwang discloses using sesquiterpene lactone compounds to inhibit NF-κB activity. Baba suggests using 1,2,3,4-tetrahydroisoquinoline compounds to treat a large number of diseases, including viral hepatitis and cytomegalovirus hepatitis, via inhibiting

NF-kB activity. Neither Hwang nor Baba teaches using any sesquiterpene lactone compound to treat HCV infection.

5. In view of the teachings of Hwang and Baba and based on my scientific knowledge, I conclude that a skilled person in the art would not have expected that sesquiterpene lactone compounds taught in Hwang, which are different from 1,2,3,4-tetrahydroisoquinonline compounds taught in Baba, can be used in treating the various diseases mentioned in Baba, e.g., viral hepatitis and cytomegalovirus hepatitis, let alone HCV infection (which is not mentioned in that reference).

6. I and my associates have assessed the activity of a sesquiterpene lactone compound, i.e., parthenolide, against cytomegalovirus (CMV), which is specifically mentioned in Baba, by conducting two assays.

The procedure of the first assay follows: Vero cells were first incubated with CMV at MOI of 0.2 in MEM at 37°C for 1 h. They were then incubated in the presence or absence of 2 μM parthenolide at 37°C for 1 h. The cells were washed thoroughly to remove the non-absorbed viruses and further cultured in a fresh medium. The supernatant was collected from the cell culture at the 6th day post infection (dpi). Viral DNA in the supernatant was extracted and amplified by PCR using a CMV UL97 primer pair.

The first assay shows that parthenolide did not significantly repress CMV production at the 6th dpi. See Figure 1 (attached).

The procedure of the second assay follows: CMV-infected MRC5 cells were cultured in the presence or absence of parthenolide in the same manner as described above. Cytopathic effects (CPEs) were observed at different time points during the culture.

The second assay shows that parthenolide did not prevent the development of CPE. More specifically, the cell culture exhibited a mild CPE at the 3rd dpi, an obvious

CPE at the 4th dpi, and a severe CPE at the 6th dpi, whether parthenolide was present or not. See Figures 2-4 (also attached).

The results of the above two assays compel the conclusion that parthenolide, a sesquiterpene lactone compound taught in Hwang, cannot be used to treat CMV hepatitis disclosed in Baba.

7. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: October 19, 2009

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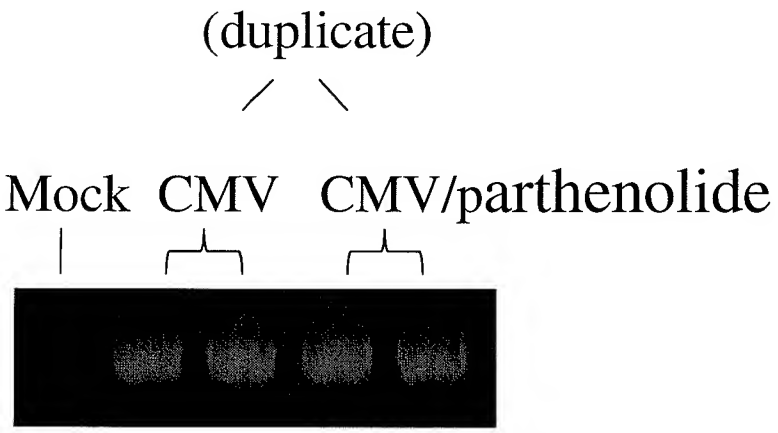


Figure 1

CMV-infected MRC5 cells (3 dpi)

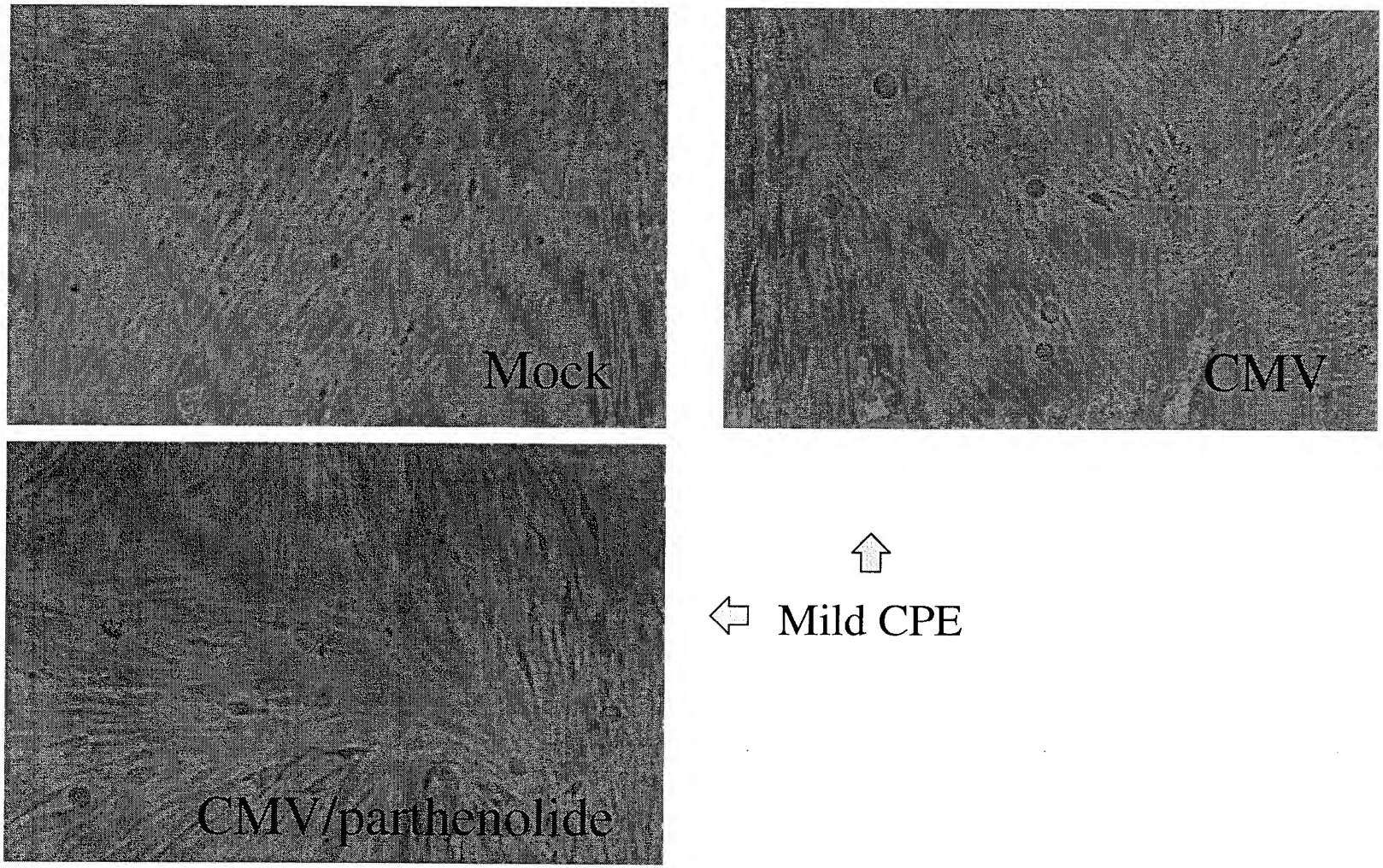
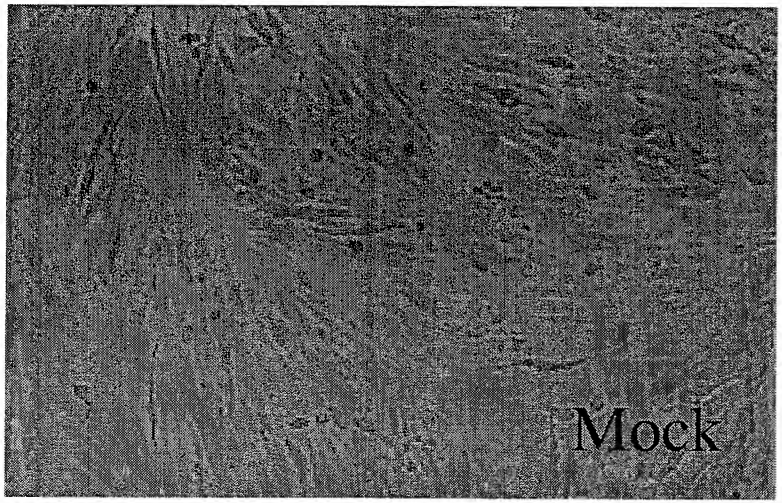
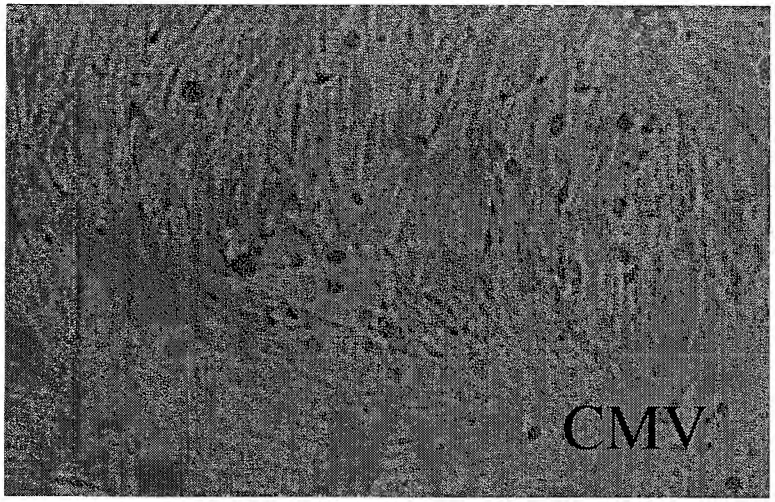


Figure 2

CMV-infected MRC5 cells (4 dpi)





⇧

⇦ Obvious CPE

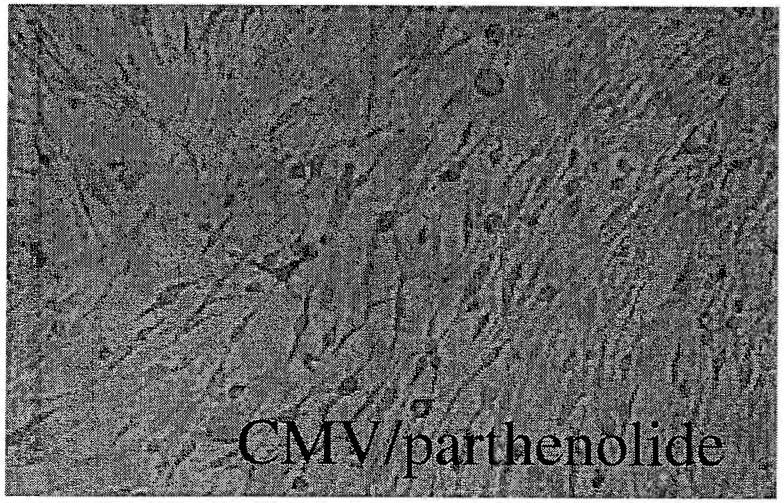


Figure 3

CMV-infected MRC5 cells (6 dpi)

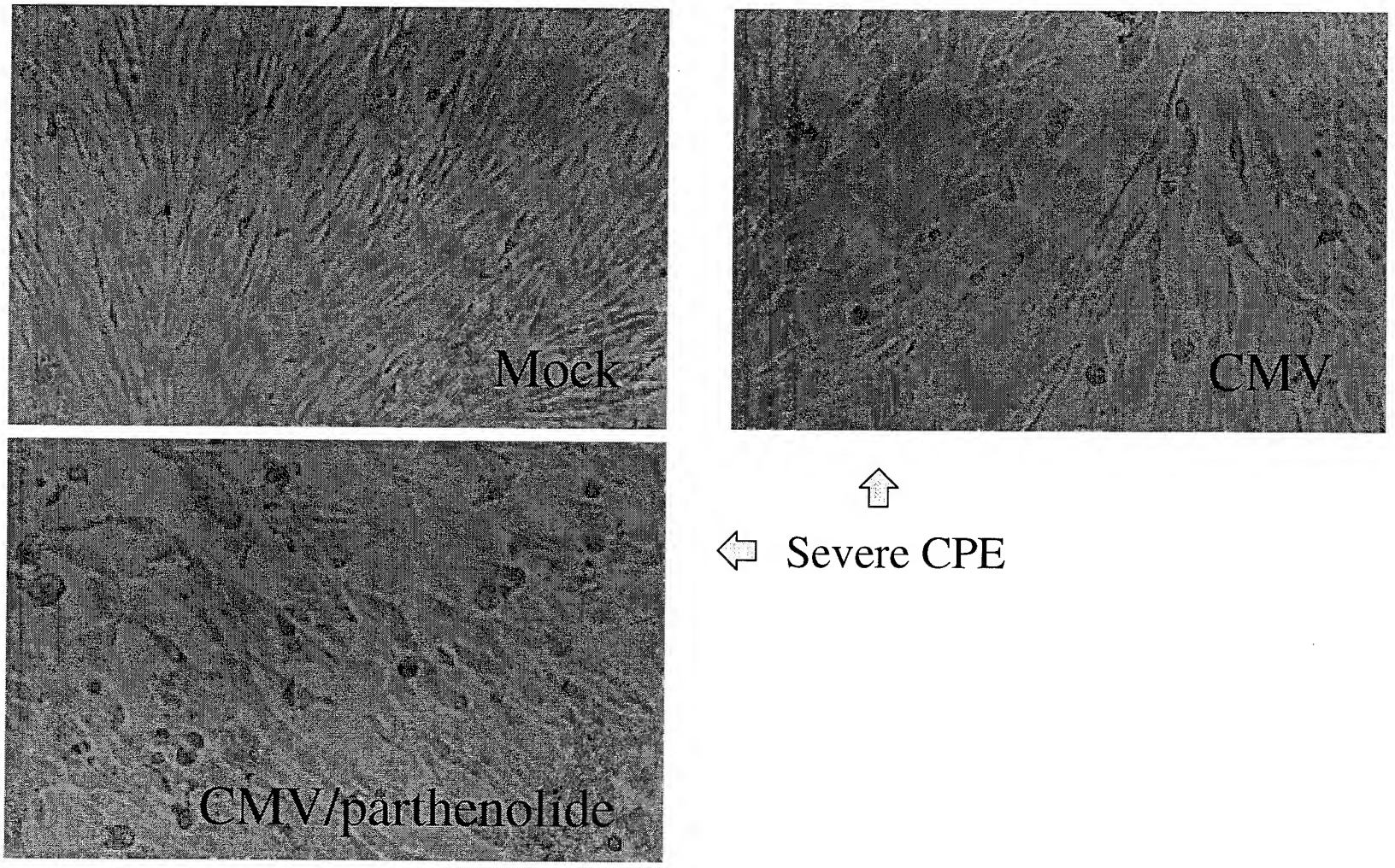


Figure 4